opportunity for us to study in this way the action of the barbiturates upon living cells, it does not seem to us that their findings conflict with the evidence here presented. Coagulation may be the mode of action within the cell, but there remains in clinical practice the all-important necessity for transporting the hypnotic from the point of administration to the interior of the cell through a series of transferences. Here, surface tension, absorption and more particularly lipoid solubility appear to be the limiting factors in the chain of events.

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Adsorption Experiments with Vitamins B(B_1) and G(B_2)

BY H. C. SHERMAN AND NELLIE HALLIDAY

Seidell's adsorption of the antineuritic vitamin upon Lloyd's reagent[1] has led to considerable experimentation with this and other forms of hydrous aluminum silicate looking to partial separation of vitamins B(B_1) and G(B_2).[2]

The experiments here recorded were planned in quantitatively graded series, and were carried out upon protein-free milk as being a material of special scientific interest in this connection because of the extent to which it has been used as a source of the vitamin-B complex in numerous researches, beginning with the work of Osborne and Mendel.[3] As adsorbent, we have used a preparation of Lloyd's reagent kindly furnished us by Professor John Uri Lloyd. This has been used in the proportions of 5, 10, 20 and 40 g. per liter of protein-free milk, prepared as described below and adjusted to P_H 3.0 and also to P_H 4.0.

The original untreated protein-free milk, the activated Lloyd's reagent and the filtrates remaining after adsorption were tested quantitatively for their vitamin B(B_1) and G potencies according to methods previously developed in this Laboratory.[4] The preparations were fed in graded amounts corresponding to definite quantities of protein-free milk or of the original skimmed milk powder. No attempt was made to study the possible supplementary relation of the two fractions, activated solids and remaining filtrates to each other. In view of the recent evidence suggesting

(1) Seidell, *U. S. Pub. Health Repts.*, **31**, 364 (1916); THIS JOURNAL, **44**, 2042 (1922); *J. Biol. Chem.*, **67**, 513 (1926).

(2) Jansen and Donath, *Proc. k. akad. wetensch. Amsterdam*, **29**, 1390 (1926); Salmon, Guerrant and Hays, *J. Biol. Chem.*, **76**, 487 (1928); *ibid.*, **80**, 91 (1928); Williams and Waterman, *ibid.*, **78**, 311(1928); Hunt, *ibid.*, **79**, 723 (1928); Guha and Drummond, *Biochem. J.*, **23**, 880 (1929); Evans and Lepkovsky, *J. Nutrition*, **3**, 353 (1930).

(3) Osborne and Mendel, Carnegie Inst. Washington, Pub. No. 156 (1911).

(4) Chase and Sherman, THIS JOURNAL, **53**, 3506 (1931); Bourquin and Sherman, *ibid.*, **53**, 3501 (1931).

the multiple nature of both the more heat labile and the more heat stable components of the vitamin-B complex and in view of the further investigations of Halliday and of Stiebeling,[5] it is conceivable that some part of our apparent losses in vitamin potencies might possibly be due to a partial separation of the component factors of what are here treated as entities, *i. e.*, vitamins B and G, respectively.

This would not change the general bearings of our findings, but might influence slightly the details of the numerical data or of graphs plotted therefrom. For this reason and in the interest of brevity, the details are omitted from this paper. They may be consulted, if desired, in the privately printed dissertation of the junior author, copies of which have been distributed by Columbia University to a number of other university libraries.

Experimental Procedure

The protein-free milk was prepared as follows. A weighed quantity of skimmed milk powder was thoroughly mixed with eight times its weight of distilled water. To this mixture was added sufficient 0.3 *M* hydrochloric acid (usually 175 cc. per 100 g. of the milk powder) to completely precipitate the casein. After filtration through several layers of cheesecloth, the resulting filtrate was heated to boiling for five minutes to precipitate the heat coagulable protein and filtered through filter paper. The filtrate or protein-free milk had a reaction as determined by electrometric measurements using a hydrogen electrode and saturated calomel half-cell, of about P_H 4.2.

The protein-free milk volume was measured and divided into three aliquots; one aliquot after being adjusted to about P_H 5.7 with 0.2 *M* sodium hydroxide was without further treatment fed as vitamin B(B_1) and as vitamin G supplements; a second aliquot after being adjusted to P_H 3.0 with 0.3 *M* hydrochloric acid was further divided and each portion treated with a definite amount of Lloyd's reagent; the third aliquot was adjusted to P_H 4.0 previous to treatment with the different amounts of Lloyd's reagent. The Lloyd's reagent was added to portions of the protein-free milk adjusted to P_H 3.0 and (or) P_H 4.0 in ratios of 40, 20, 10 and 5 g., respectively, per liter of the protein-free milk. The adsorbent and solutions, after thorough mixing, were allowed to stand in contact in a refrigerator overnight. The "activated solids" were then separated by use of a Büchner funnel, washed with dilute hydrochloric acid (P_H 3.0 or P_H 4.0), dried at room temperature for twenty-four hours, pulverized and used thus as vitamin B(B_1) and as vitamin G supplements for the experimental animals. Each filtrate remaining after adsorption was evaporated under reduced pressure (30–40°) to approximately one-fourth of its original volume, adjusted to about P_H 5.7 and the respective volumes measured in order that known equivalents in terms of the protein-free milk could be fed for measurements of their vitamin B(B_1) and vitamin G potencies.

These several preparations were kept in an ice-box and freshly prepared each week. As supplements the liquid preparations were fed in six equal amounts weekly, the solid preparations were incorporated in the basal rations each week using such quantities of the basal ration for this as would be somewhat less than the animal would eat during the week as judged by previous weekly food records, basal diet alone being fed for the remainder of the week. The experimental animals were in all cases weighed weekly.

Results

Animals receiving the original skimmed milk powder and animals receiving corresponding amounts of untreated protein-free milk as sources

(5) Halliday, *J. Biol. Chem.*, **96**, 479 (1932); Stiebeling, *Proc. Soc. Exptl. Biol. Med.*, **29**, 1155 (1932).

of vitamin B(B_1) showed practically the same average responses in growth, indicating little or no loss of potency in the preparation of the protein-free milk. The same was true as regards the vitamin G content of the protein-free milk.

When the amount of Lloyd's reagent employed was 40, 20, 10 or 5 g. per liter of protein-free milk, the filtrates were without appreciable vitamin B(B_1) activity; approximately 50% of the original vitamin B(B_1) potency was usually accounted for in the activated solids. Whether the protein-free milk had been adjusted to P_H 3.0 or to P_H 4.0 previous to the addition of the adsorbent made no significant difference in the vitamin B or in the vitamin G potencies of the activated solids or filtrates. In all cases the difference between the average growth rates of animals receiving, respectively, the product prepared from protein-free milk adjusted to P_H 3.0 and that adjusted to P_H 4.0 was less than the probable error of that difference. The addition of the adsorbent caused a decrease in the acidity of the solutions, and this was more marked with the larger amounts of adsorbent. When the adsorption processes were carried out in an atmosphere of nitrogen rather than air, the activated solids instead of showing only 50% of the original vitamin B(B_1) of the protein-free milk showed from 60 to 70% of the potency. The activated solids carried about one-third of the vitamin G potency of the protein-free milk and the filtrates regularly contained about one-sixth of this original potency, so that in all 50% of the vitamin G activity of the protein-free milk could be accounted for after the fractionation by adsorption with Lloyd's reagent.

When the adsorption processes were carried out in an atmosphere of nitrogen both activated solids and filtrates showed somewhat higher vitamin G potency than when the adsorption was carried out in air, the recovery under nitrogen being about two-thirds as compared with about one-half in the presence of air. The nature of these apparent losses is being studied further.

Summary

1. Protein-free milk may be prepared from skimmed milk powder without appreciable loss of vitamin B(B_1) or vitamin G(B_2) potency.

2. Vitamin B(B_1) is relatively more efficiently adsorbed from protein-free milk on Lloyd's reagent than is vitamin G.

3. Varying the amount of Lloyd's reagent used from 5 to 40 g. per liter of protein-free milk (initially adjusted to P_H 3.0 or P_H 4.0) did not result in appreciable differences in the amounts of vitamin B(B_1) or vitamin G(B_2) adsorbed.

4. Under the conditions of these experiments approximately one-half of the vitamin B(B_1) potency and one-third of the vitamin G potency of the original protein-free milk was adsorbed on the Lloyd's reagent; but no appreciable amount of the original vitamin B(B_1) potency and only about

one-sixth of the vitamin G potency was shown by the filtrates after removal of the activated solids.

5. When the adsorption processes were carried out in an atmosphere of nitrogen rather than air and under otherwise comparable conditions, the activated solids were somewhat more potent in both vitamins B(B_1) and G and the filtrates were more potent in vitamin G. The apparent losses are being studied in other ways and in the light of the newer evidence as to probable multiple nature of vitamins B and G.

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Liquid Ketene and Ketene Polymers

BY CHARLES D. HURD, AMOS D. SWEET AND CHARLES L. THOMAS

In a study of liquid ketene which was prepared by liquefaction from the gas stream from the pyrolysis of acetone, a few new items of interest were found. (1). Polymerization, which occurs even at −80°, may be retarded by a trace of hydroquinone (2 mg. for 2 cc. of liquid). (2). No reaction was observed in twelve hours between liquid ketene and metallic sodium or sodium–potassium (1:2) alloy or sodamide (suspended in toluene) or isoprene. Thus, the alkali metals did not bring about rearrangement of ketene into hydroxyacetylene (HC≡COH) and the isoprene did not add in the way it adds to maleic anhydride.[1] Possibly the low temperature was a contributing factor in these negative effects. (3). An extremely vigorous reaction was observed between liquid ketene and the Grignard reagent. Even at −78° and diluted with ether the reaction with *n*-butyl- or phenylmagnesium bromide was so vigorous that each added drop of Grignard reagent produced a crackling sound. The voluminous yellow solid which precipitated hydrolyzed to a sticky gum from which nothing was obtained. This is a modification of the experiment of Deakin and Wilsmore.[2] They diluted gaseous ketene with hydrogen and observed a very complex reaction as it was passed into a cold ether solution of methylmagnesium bromide.

(4). Tertiary alcohols react with liquid ketene giving *tert*-alkyl acetates. In previous unpublished work,[3] it was demonstrated that ketene gas also acetylates these tertiary alcohols but less effectively. This confirms Davis and Murray[4] who stated: "Tertiary butyl acetate, a typical tertiary ester, is suitably prepared by the action of ketene with tertiary butanol." However, Davis and Murray cited no experimental work.

(1) Diels and Alder, *Ann.*, **460**, 98 (1928); **470**, 62 (1929); **478**, 137 (1930).
(2) Deakin and Wilsmore, *J. Chem. Soc.*, **97**, 1968 (1910).
(3) Hurd and Martin, M.S. thesis of K. E. Martin, Northwestern University, 1928.
(4) Davis and Murray, *Ind. Eng. Chem.*, **18**, 846 (1926).